REMARKS/ARGUMENTS

Claims 1-4, 10 and 11-21 are active (products and methods of making product). Claims 5-9 are directed to a method of use and have been withdrawn from consideration. The specification has been revised to disable active hyperlinks. The claims have been amended for consistency with U.S. practice by adding the term "isolated or purified" and by further structural definition of genetic components by reference to sequence identifiers. The *sig A* sequence is structurally described by reference to SEQ ID NO: 1; see page 9, [0019] for support. The *Bacillus* promoters for *sigH* and for the *spoIIA* operon appearing in claim 1 are described in the paragraph bridging pages 8-9, pages 10-11 and on pages 20-21 of the specification. Moreover, *Bacillus* sporulation genes and their promoters were well-known as shown by Kroos, et al., Mol. Microbiol. 31:1285 (attached) and Dubnau, et al., J. Bacteriol. 170:1054 (attached) and Sung, et al., Gene 194:25-33 (attached, abstract only). These publications show that *spo0H* (*sigH*) and *spoIIA* (*sigF*) are present in *Bacillus* other than *Bacillus subtilis* (Dubnau, et al. and Sung, et al.), that these genes are expressed during an early sporulation stage (Kroos, et al.), and that *sigH* and *spoIIA* genes were known to express during an early sporulation stage in *Bacillus*, a sporogenous bacterium which has a promoter sequence recognized and transcribed specifically during a sporulation stage, such as *sigH* or *spoIIA* or analogs thereof.

Claim 2 specifically refers to *Bacillus subtilis sigH* and *spoIIA* operon promoters that are structurally identified on pages 20-21 by reference to SEQ ID NOS: 2 and 3. Claim 10 has been amended for consistency with claim 1. Claims 14 and 15 find support on page 8 at paragraph [0017]. New claims 11-20 find support as for claims 1 and 10 and in the original claims and specification. Claim 21 finds support at paragraph [0013] of U.S. 2009/017054. No new matter has been added. Favorable consideration of this amendment and allowance of this case are respectfully requested.

The Applicants thank Examiner Lee for the courteous and helpful interview of April 14, 2010. It was suggested that the Applicants uniformly use either the term "DNA" or "polynucleotide" in the claims. To avoid a potential enablement issue, the Examiner suggested imposing a functional limitation on the phrase "at least 70% homologous to SEQ ID NO: 1", such as specifying that this polynucleotide encodes a SigA polypeptide. The Examiner agreed to consider claim language that structurally defined a genus of sequences at least 70% homologous to SEQ ID NO: 1, but indicated that this language may raise enablement or prior art issues.

It was suggested that the Applicants avoid the phrase "sequence equivalent thereto" to avoid an issue of indefiniteness with regard to how an equivalent sequence was determined. To address the anticipation rejection, it was suggested that the claims be limited to structurally defined promoter sequences and that the Applicants explain how these promoter sequences differ from those of Hicks, et al. The Examiner also suggested that the Applicants explain why Hicks would not render the invention obvious.

Priority/Information Disclosure Acknowledgments

The Applicants thank Examiner Lee for acknowledging their priority claims and the documents cited on Forms 1149 returned with the Official Action. The OA refers to lined-through references, however, none were evident in the returned Forms 1449, so the Applicants understand that all of the cited documents have been considered.

Restriction/Election

The Applicants previously elected with traverse **Group I**, claims 1-4 and 10, directed to a mutant *Bacillus* bacterium. The Applicants respectfully request that the claims of the nonelected group(s) or other withdrawn subject matter which depend from or otherwise

include all the limitations of an allowed elected claim, be rejoined upon an indication of allowability for the elected claim, see MPEP 821.04. The Applicants express their gratitude to Examiner Lee for confirming on page 5 of the OA that the method of use claims, claims 5-9, are subject to rejoinder.

Objections—Specification and Claim 4

These objections are moot in view of the amendments above. No revision was made to page 8 of the specification for the purpose of identifying "Genetyx-Win" as a trademark since a search of TESS (Trademark Electronic Search System) did not identify this term as a trademark.

Rejections—35 U.S.C. §112, second paragraph and 35 U.S.C. §101

Claims 1-3 and 10 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite and under 35 U.S.C. 101 as being non-statutory claims. This rejection is moot in view of the amendments above.

Rejections—35 U.S.C. §112, first paragraph

Claims 1-3 and 10 were rejected under 35 U.S.C. 112, first paragraph, as lacking adequate written description and adequate enablement. These rejections are moot in view of the amendments above which remove functional terms and describe the components of the invention structurally or by reference to sequence identifiers.

Rejection—35 U.S.C. §102

Claims 1-3 and 10 were rejected under 35 U.S.C. §102(b) as being anticipated by Hicks, et al., Mol. Microbiol. 20:201, in light of Haldenwang, Microbiol. Rev., March 1995 (applied as an evidentiary reference). This rejection is moot in view of the amendments above. Hicks was cited as teaching wild-type *Bacillus subtilis* strains JH642b and KH441c and mutant strains KH311b and KH516c. The invention requires a promoter sequence "located in a region of **1 to 198 bp upstream** of, and operatively-linked to, said polynucleotide encoding *sigA*". The two wild-type strains do not read on the invention because none of the P1-P6 promoters shown in Fig. 1 of Hicks meet the above limitations of claim 1. The P3 and P4 promoters which Hicks indicates are controlled by *sigH* (see legend to Fig. 1 and page 202, col. 1, lines 13-14) are not 1 to 198 bp upstream of *sigA* (*rpoD* in Fig. 1 of Hicks).

Furthermore, the Examiner has not demonstrated that these promoters are operatively-linked to *sigA* (*rpoD*), especially in view of the intervening *dnaG* (DNA primase) sequence. P1 and P2 are controlled by *sigA* and the sigma factor that controls P5 is unknown (Hicks, legend to Fig. 1). P6 which is upstream of *sigA* (*rpoD*) in Hicks is controlled by *sigD*. Therefore, the wild-type strains of Hicks JH642b and KH441c do not fall within claim 1.

The two mutant strains of Hicks KH311b and KH516c have an IPTG-inducible promoter *Pspac*, see page 205 of Hicks, Table 1. According to El-Gawily, *Biotechnology Annual Review*, vol. 1, Elsevier Science (1995), pp. 122-123:

Pspac promoter

The Pspac promoter represents an elegant example of a genetically engineered pro-

moter that is efficient and controllable and capable of expressing foreign genes [41,42]. This hybrid promoter is a derivative of a promoter of the *B. subtilis* phage SPO1 and the operator site of the *E. coli lac* gene. The controlled expression of Pspac is dependent on the simultaneous presence of the *lac* operator that lies between the SPO1 promoter and the *B. subtilis* ribosome binding site and the *E. coli* repressor gene, *lacI*, that was engineered to be expressed in *B. subtilis*. This was done by substituting the *B. licheniformis* promoter and RBS of the penicillinase gene for the *E. coli lacI* gene promoter and RBS. The presence of the *lac* repressor in *B. subtilis*, in the absence of a *lac* inducer, prevented the expression of the hybrid Pspac promoter. The addition of isopropyl-β-thiogalactoside (ITPG) allowed expression from the Pspac promoter.

This whole construct of the Pspac promoter followed by a *lac* operator site, ribosome binding site, initiation codon and a foreign gene and the *lac* repressor gene was inserted into a plasmid shuttle vector capable of replicating in both *E. coli* and *B. subtilis*. With these constructs it was found that the promoter was indeed regulated by the *lac* operator/repressor system and that foreign gene expression could be controlled by the presence and absence of the inducer for this system.

The two mutant strains of Hicks employ this promoter which is not a promoter for *sigH* as defined by claim 1.

Hicks would also not render the invention obvious, because it does not disclose the promoter sequences described by reference to SEQ ID NOS: 2 and 3 and is thus does not teach an essential element of the invention. Hicks also provides no motivation for selecting a promoter for *sigH* or for the *spoIIA* operon to control expression of the SigA protein during particular stages of the *Bacillus* sporulation. In contrast, the inventors have shown that selection of these promoters to provide a stage specific increase in expression of SigA significantly improves the expression of protein or polypeptides from a heterologous gene in by recombinant *Bacillus*, see e.g., the top of page 8 of the specification. The native promoters, such as P1-P6 of Hicks would not improve the expression of a heterologous gene since they act in the natural sequence. The *Pspac* promoter in the mutant strains of Hicks does not provide the stage-specific expression of SigA because this promoter requires a *lac*

inducer such as IPTG. In view of these differences between the Hicks strains and the *Bacillus* strains encompassed by independent claim 1, this rejection cannot be sustained.

Conclusion

This application presents allowable subject matter and the Examiner is respectfully requested to pass it to issue. The Examiner is kindly invited to contact the undersigned should a further discussion of the issues or claims be helpful.

Respectfully submitted,

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